



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 5009.209-2016

**National food safety standard - Determination of Zearalenone in
Cereals**

食品安全国家标准 食品中玉米赤霉烯酮的测定

(English Translation)

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1 Scope

This Standard specifies methods for the determination of zearalenone in foods.

The method 1 of this Standard is applicable to the determination of zearalenone in food and food products, alcohol, soy sauce, vinegar, sauce and sauce products, soybean, rapeseed and edible vegetable oil; method 2 is applicable to the determination of zearalenone in soybean, rapeseed and edible vegetable oil; method 3 is applicable to the determination of

zearalenone in beef, pork, beef liver, milk and egg.

Method 1 -- Liquid Chromatography

2 Principle

Use acetonitrile solution to extract zearalenone in the sample; after it is purified by the immunoaffinity column, use high-performance liquid chromatography fluorescence detector to measure; use external standard method to quantify.

3 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, the water is grade-1 water specified by GB/T 6682.

3.1 Reagents

3.1.1

Methanol (CH₃OH)

Chromatographic pure.

3.1.2

Acetonitrile (CH₃CN)

Chromatographic pure.

3.1.3

Sodium chloride (NaCl).

3.1.4

Potassium chloride (KCl).

3.1.5

Disodium hydrogen phosphate (Na₂HPO₄).

3.1.6

Monopotassium phosphate (KH₂PO₄).

glass-fiber filter paper to filter it until the filtrate is clarified; reserve the filtrate for later use.

5.1.4 Alcohol

Take 20.0 g of degassed alcohol sample (alcohols which contain carbon dioxide, before using, shall be placed in a refrigerator at 4°C for 30 min, and filtered or ultrasonically degassed) or other alcohol samples which do not contain carbon dioxide in a 50 mL volumetric flask; use acetonitrile to fix-volume to the scale;

shake well. Pipette 10.0 mL of the filtrate; add 40 mL of water; dilute and mix;

use a glass-fiber filter paper to filter it until the filtrate is clarified; reserve the filtrate for later use.

5.2.1 Food and food products

Connect the immunoaffinity column to a glass syringe; accurately transfer 10 mL (equivalent to 0.8 g of samples) of the filtrate in 5.1; inject into the glass syringe. Connect the air pressure pump to the glass syringe; adjust the pressure, so that the solution passes through the immunoaffinity column at a flow rate of

1 drop/s ~ 2 drops/s, until part of the air enters the affinity column. Use 5 mL of water to rinse the column for one time, at a flow rate of 1 drop/s ~ 2 drops/s, until part of the air enters the affinity column; discard all the effluent. Accurately add 1.5 mL of methanol to elute at a flow rate of about 1 drop/s. Collect the eluate in a glass test tube; use nitrogen to dry at 55°C or lower; then, use 1.0 mL of the mobile phase to dissolve the residue, which is for liquid chromatography determination.

5.2.2 Soy sauce, vinegar, sauce and sauce products, alcohol

Connect the immunoaffinity column to a glass syringe; accurately transfer 10.0 mL of the filtrate in 5.1.2 or 5.1.4; inject into the glass syringe. Connect the air pressure pump to the glass syringe; adjust the pressure, so that the solution slowly passes through the immunoaffinity column at a flow rate of 1 drop/s ~ 2 drops/s, until part of the air enters the affinity column. Successively use 10 mL of PBS washing buffer and 10 mL of water to rinse the immunoaffinity column, at a flow rate of 1 drop/s ~ 2 drops/s, until the air enters the affinity column;

discard all the effluent. Accurately add 1.0 mL of methanol to elute at a flow rate of about 1 drop/s. Collect the eluate in a glass test tube; use nitrogen to dry at

55°C or lower; then, use 1.0 mL of the mobile phase to dissolve the residue, which is for liquid chromatography determination.

5.2.3 Soybean, rapeseed, edible vegetable oil

Connect the immunoaffinity column to a glass syringe; accurately transfer 10 mL of filtrate in 5.1.3; inject into the glass syringe. Connect the air pressure

Method 2 -- Fluorescence spectrophotometry

9 Principle

Use acetonitrile solution to extract zearalenone in the sample; purify through the immunoaffinity column; then, add aluminum chloride solution for derivation.

Use a fluorometer to determine the eluent.

10 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, the water is grade-1 water specified by GB/T 6682.

10.1 Reagents

10.1.1

Acetonitrile (CH_3CN)

Chromatographic pure.

10.1.2

Methanol (CH_3OH)

Chromatographic pure.

10.1.3

Sodium chloride (NaCl).

10.1.4

Disodium hydrogen phosphate (Na_2HPO_4).

10.1.5

Monopotassium phosphate (KH_2PO_4).

10.1.6

Potassium chloride (KCl).

10.1.7

Tween-20 ($\text{C}_{58}\text{H}_{114}\text{O}_{26}$).

10.1.8

Hydrochloric acid (HCl).

10.1.9

Sulfuric acid (H_2SO_4).

10.1.10

Aluminum chloride ($\text{AlCl}_3 \cdot 10\text{H}_2\text{O}$).

10.1.11

Quinine sulfate ($\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2 \cdot \text{H}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$).